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**EFFECTS OF HIGH-ENERGY X-RAYS ON THE
REPRODUCTION OF UROSALPINX CINEREA**

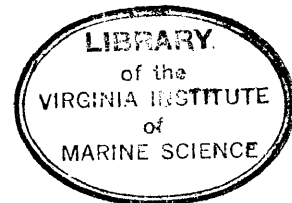
by

KENNETH A. LEON

A THESIS

**Submitted to the School of Marine Science
of the College of William and Mary
in partial fulfillment of the requirements
for the degree of
MASTER OF ARTS**

1963



APPROVED

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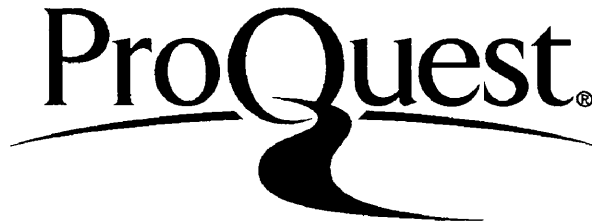
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ABSTRACT

The oyster drill, Urosalpinx cinerea, was subjected to x-ray dosages of 3000, 9000, and 18,000 roentgens to determine the effects on reproduction.

A dose of 18,000r applied to male drills significantly reduced the viability of their offspring in two of the experiments. Irradiated females produced either a relatively small number of egg cases or no egg cases at all. They were apparently stimulated to produce the bulk of their total production in a much shorter time than the control females.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	
INTRODUCTION	1
METHODS AND MATERIALS	3
RESULTS	9
General considerations	9
Experiment 1	9
Experiment 2	14
Experiment 3	18
DISCUSSION	23
SUMMARY	26
LITERATURE CITED	27
APPENDIX	28

LIST OF TABLES

1. Viability and egg case - embryo totals (Experiment 1)	12
2. Egg case production with time (Experiment 1)	13
3. Viability and egg case - embryo totals (Experiment 2)	16
4. Egg case production with time (Experiment 2)	17
5. Viability and egg case - embryo totals (Experiment 3)	21
6. Egg case production with time (Experiment 3)	22

LIST OF FIGURES

IN TEXT

	Page
1. Top. Filtered running water apparatus Bottom. Tube for holding egg cases	7
2. Embryo development stages	8

IN APPENDIX

3. Embryo survival (Experiment 1)	28
4. Embryo survival (Experiment 2)	29
5. Embryo survival (Experiment 3)	30
6. Per cent of total egg case production with time (Experiment 1)	31
7. Per cent of total egg case production with time (Experiment 2)	32
8. Per cent of total egg case production with time (Experiment 3)	33

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Introduction

It is well known that x-rays can induce structural changes in the chromosomes of many organisms. Bushland and Hopkins (1953) used this principle in their researches involving the sterilization of the screw-worm fly, Callitroga hominivorax, a parasite of cattle, sheep, goats, and horses. First, they determined that high-energy x-rays produced dominant lethal effects in the screw-worm fly. Then they found that the sperm of irradiated male flies carrying this lethal effect competed equally with the sperm of non-irradiated males. Subsequently, millions of hatchery-propagated flies were irradiated and released into the field. Yearly introductions of these irradiated flies among the natural populations of Curacao and Florida reduced the numbers of viable embryos until the pests were eliminated (Bushland, 1960).

Since this irradiation technique worked so well on the screw-worm fly, it occurred to Dr. William J. Hargis, Jr. in 1955 (Hargis, personal communication) that the principle might be applied to the elimination of a serious pest of the Atlantic coastal waters of the United States, the oyster drill, Urosalpinx cinerea.

Several questions had to be answered before the feasibility of such an irradiation control procedure could be determined. First, the LD₅₀ had to be established in order that the range of possible sublethal doses could be determined. This was done in a series of experiments described by Hargis et al. (1957) in which the LD₅₀ was

established at about 21,000 to 24,000 roentgens. Three problems which remained unsolved were:

1) whether or not x-rays could produce lethal effects in the sperm of male oyster drills, i.e. sterilize them.

2) whether the dosages necessary to produce such changes in the chromosomes would be damaging to the health and vitality of the irradiated adults. Obviously, irradiated males would have to be healthy enough to inseminate the females of the natural population in competition with non-irradiated males.

3) whether sperm from irradiated males would compete equally with the sperm from normal males in the reproductive tract of the female, since Hargis and MacKenzie (1961) established that both sexes of this species are promiscuous and that sperm are retained for long periods of time by the females. Only with such competition would a mathematical possibility of elimination exist.

The research reported herein was designed by the author and Dr. Hargis and carried out by the author in order to seek out answers to these questions.

Methods and Materials

Three experiments were conducted; one during the spring egg deposition period of 1962, one during the fall deposition period of the same year, and the last during the spring egg deposition period of 1963. All specimens were collected from eel grass zones of the York River, in the vicinity of the Virginia Institute of Marine Science. Drills were hand picked from the eel grass, pilings, and bottom. Animals for the first experiment were collected between May 1 and May 11, for the second experiment between August 5 and August 12, and for the third — between April 15 and April 24.

Sex of individual snails was determined by the presence or the absence of a penis. This was accomplished by using a modification of the technique developed by Hargis (1957) for sexing gastropods. Submerged drills were held against the bottom inside edge of a Petri dish until they attached to the glass. Then, with the shell rolled back, the right dorsal side of an animal just behind the tentacles was examined with a stereoscope for the presence of a male organ.

After being sexed, the shells of all experimental animals were cleansed with a toothbrush and marked with paint, the males with red and the females with yellow.

In all experiments, 150 drills of each sex were used. Only those of a size considered sexually mature (Hargis-Whitcomb, personal communication) were used. Male drills were from 13mm to 21mm in length, with a mean of 15.6mm. Female drills were from 14mm to 22mm, with a mean of 17.2mm. The various sized drills were later divided among the

experimental containers so that no mean difference in drill length existed from container to container.

In each experiment, the drills were wrapped in moist cheesecloth and transported to the Biophysics Department of the Medical College of Virginia for irradiation. Free evaporation from the periodically moistened cheesecloth prevented possible mortality due to high temperature.

At the radiation laboratory, the drills were placed in rectangular plastic containers approximately 2 inches by 3 inches by 1 inch in size. Rubber bands were used to hold the drills stationary and in proper position. A backing of wet filter paper prevented a loss of moisture from the irradiation containers. The plastic containers were then fastened around the periphery of a horizontal turntable in a circle. The turntable was rotated at 45 RPM, 36 inches from the source. The 1000 KVP machine produced a wide beam which covered the containers from the horizontal. A thimble chamber rotated under the same conditions measured the dosages. There were no soft components of the beam because the water jacket had already filtered them out. Under these conditions the half-value layer in lead was 3.75mm. A dose rate of 576 roentgens per minute was administered by the physicist in charge, Harry Muller.

In each experiment, three groups of drills, each consisting of 30 males and 30 females, were irradiated. The first group received a total dosage of 3000r; the second group, 9000r; and the third, 18,000r. Control drills were held in containers under similar conditions, but were not irradiated.

After the animals were returned to the Institute, they were divided equally among three continuous flow aquaria. In each aquarium, ten polyethelene containers were used to hold the drills. The perforated containers measured approximately 4 inches by 4 inches by 3 inches. Each held five male and five female drills.

Of the ten containers in each aquarium, one contained non-irradiated males and females as a control group. Three others in each aquarium held non-irradiated females but differed from one another in that one held males which had received 3000r, another held males subjected to 9000r, and the third — males which had received 18,000r. A second group of three containers in each aquarium held females irradiated at 3000, 9000, and 18,000r, respectively, accompanied by non-irradiated males. The third group of three containers in each aquarium held both males and females irradiated at 3000, 9000, and 18,000r, respectively. As there were five males and five females in each container, and each group was triplicated, a total of 15 pairs comprised each dosage combination. In experiments 1 and 2, 15 pairs of control drills were used, whereas 30 pairs were used in the third.

Periodic switching of containers from one aquarium to another and random positioning of containers within each aquarium is believed to have eliminated location and position differences which might have increased difficulties in statistical analysis.

Throughout the experiments, small fresh Mya, Mulinia, or Crassostrea were put in each container for food. The food animals

were removed when their valves opened enough to indicate that they were dying. York River water, with salinity ranging from about 14 to 20 parts per thousand, or the same that the drills encounter in nature, was continuously filtered through layers of gravel, fine sand, and glass wool, and passed into each aquarium. For the last experiment, more refined and efficient filters of glass wool were constructed. Filters, as well as aquaria, were cleaned periodically.

Each container was examined for egg cases approximately four times a week. When it appeared that a female had finished depositing a cluster of eggs, the cluster was carefully removed from the substrate with a scalpel and placed in a glass tube (Fig. 1), 5cm long and 12mm in diameter. To keep the egg cases from washing out of the tubes, small squares of nylon stocking material were secured to the ends of the tubes with rubber bands. The tubes were numbered for identification and placed in a special holding apparatus supplied with filtered running water (Fig. 1).

Subsequently, daily microscopic examination of the egg cases were made in which the number of embryos, the degree of development (Fig.2), and viability as indicated by movements of the embryos, were noted. When the embryos in an egg case died, the latest stages of development attained were recorded, the entire egg case was fixed in a Bouin's solution and preserved in 70 per cent alcohol.

Figure 1. Top. Filtered running water apparatus.

Bottom. Tube for holding egg cases.

Figure 1

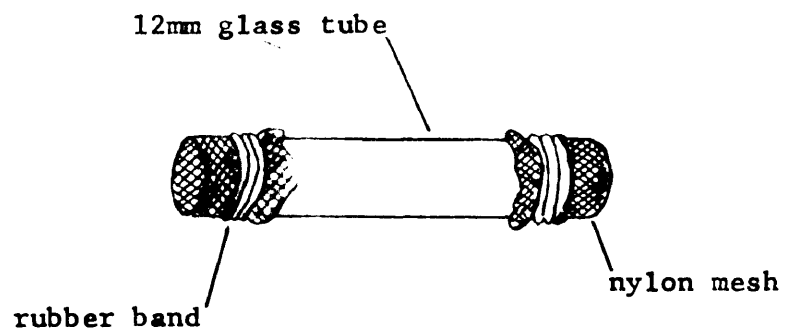
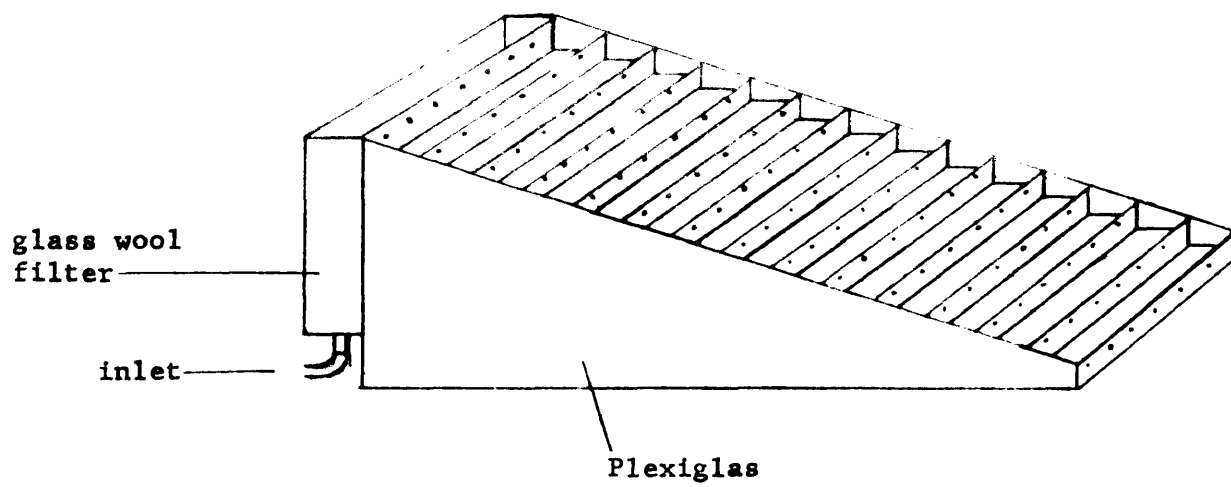
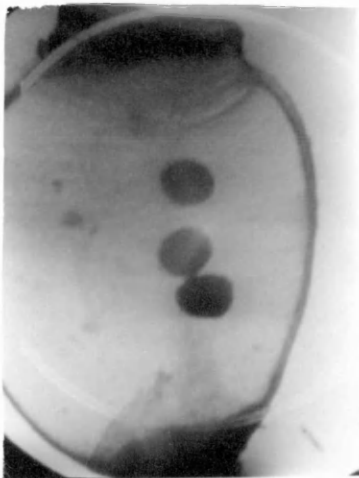


Figure 2. Embryo development stages.

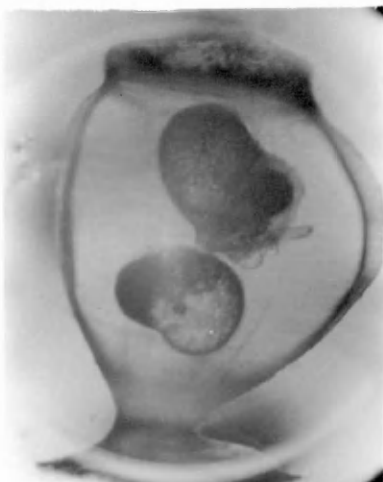
Figure 2



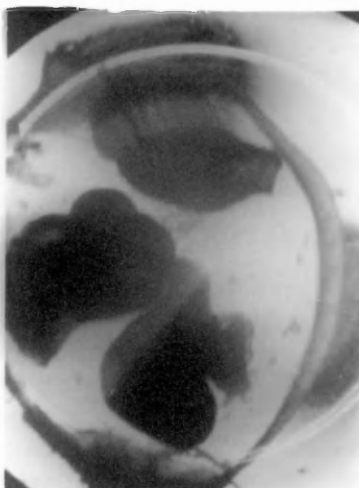
A. Blastulae



B. Gastrulae



C. Veligers



D. Protoconchs



E. Abnormal

Results

General Considerations

Before discussing the data, mention must be made concerning the effect of adult mortality in the three experiments. Since totals of egg cases produced were used as a measure of the effects of irradiation on production (fecundity), it was important to include the same number of adults in each irradiation group. When an adult female died, a potential source of production was removed from the experiment. Since male drills are promiscuous, their mortality probably had little effect on production during the experiments.

A review of female mortalities indicates that only in the control group of experiment 2, where two females died on the first day, could production have been altered to any extent. In all other cases, only once did more than one female die. This mortality, consisting of two females, occurred on the last day of the experiment.

Experiment 1

The first experimental group was irradiated on May 22, 1962. Observations of the drills prior to irradiation showed that egg case deposition had been under way for some time. Actually, egg cases were first seen on May 5. As will be shown later, this seems to have a bearing on fecundity and viability of irradiated adults.

Observations on this series were made every day for a short period and thereafter were carried out every two to three days depending on production of egg cases.

The 15 pairs of controls produced 23 egg cases containing 77 embryos. All survived to veliger and protoconch stages. As can be seen in Table 1, the three groups in which the males were irradiated at successively higher levels (i.e. 3000r, 9000r, 18,000r) all produced egg cases with viable embryos whose survival was not significantly lower than those of the controls. In other words, the fecundity of this group of snails and survival of the embryos was similar to the controls.

However, in the group in which only the females of each pair were irradiated, the results were:

- 1) 3000r - 62 egg cases containing 148 embryos were produced, with 138 or 93.2 per cent surviving to veliger and protoconch stages;
- 2) 9000r - 21 egg cases bearing 63 embryos, with 11 individuals or 18 per cent surviving; and
- 3) 18,000r - produced 20 egg cases containing 70 embryos with none surviving.

In the 15 pairs with both sexes irradiated, the results were:

- 1) 3000r - 53 egg cases bearing 184 embryos with 146 or 79 per cent surviving;
- 2) 9000r - 57 egg cases with 213 embryos and only 32 per cent surviving; and
- 3) 18,000r - 29 egg cases with 111 embryos and no survival.

It can be seen that survival of embryos was markedly lower in those pairs in which the females or both sexes received the higher doses (i.e. 9000 and 18,000r). Lesser effects were noted at 3000r.

Further examination of the data referred to above (Table 1)

indicates that under the condition of this experiment, irradiation of males alone produced no significant reduction in fecundity or viability of resulting embryos (see also Fig. 3).

This may have occurred because the experiment was conducted at a time when copulation was minimal. According to past observations, the most intense copulatory activity precedes the spring egg case deposition period, and viability of embryos could not be significantly affected by irradiation of males alone because their sperm did not enter into the process.

An interesting result was that egg case production of irradiated females is probably affected by irradiation (Fig. 6). Irradiated females were apparently stimulated in such a way that they produced most of all the egg cases they were to produce for the duration of the experiment much more rapidly than those groups whose females were not irradiated (see also Table 2).

This experiment was terminated earlier than had been planned because of water system failure.

Table 1

Viability and Egg Case - Embryo Totals

Experiment 1

<u>Mated Pairs</u>	<u>Total Egg Cases</u>	<u>Total Embryos</u>	<u>% Reaching Veliger & Protoconch</u>
15 ♂ 3000r. 15 ♀ 0	13	31	100.0
15 ♂ 9000r. 15 ♀ 0	69	225	93.3
15 ♂ 18000r. 15 ♀ 0	49	175	94.9
15 ♂ 0 15 ♀ 3000r.	51	148	93.2
15 ♂ 0 15 ♀ 9000r.	21	63	17.5
15 ♂ 0 15 ♀ 18000r.	20	70	0.0
15 ♂ 3000r. 15 ♀ 3000r.	62	178	80.3
15 ♂ 9000r. 15 ♀ 9000r.	57	213	31.9
15 ♂ 18000r. 15 ♀ 18000r.	29	111	0.0
15 ♂ control 15 ♀ control	23	77	100.0

Table 2

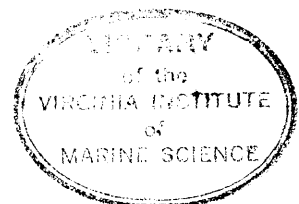
Egg Case Production With Time

Experiment 1

Date	X ♂♂' & N ♀♀			N ♂♂' & X ♀♀			X ♂♂' & X ♀♀			Control		
	No. Cases	Accum. %		No. Cases	Accum. %		No. Cases	Accum. %		No. Cases	Accum. %	
May 26, 1962	2	1.5		10	10.9		32	21.6		-	-	
27	3	3.8		-	10.9		51	56.1		-	-	
29	47	39.7		80	97.9		43	85.2		13	56.5	
June 2	37	67.9		1	99.0		6	89.3		3	69.5	
6	42	100.0		1	100.0		16	100.0		7	100.0	

X - Irradiated

N - Non-irradiated



Experiment 2

A second experiment, planned at the outset of this research, proved doubly necessary due to the failure of the water system during experiment 1, and also because we wished to compare the results of an experiment begun after the early summer egg case deposition period had terminated. The second series was irradiated on August 20, 1962. Observations were made every day until November 2, 1962 or 74 days after irradiation, when cold water temperatures brought embryo development to a halt.

As can be seen in Table 3, the non-irradiated pairs in this series (controls) produced 30 egg cases bearing 74 embryos. Eighty-two per cent (61) survived to the veliger and protoconch stages and/or hatched.

As in experiment 1, the pairs in which only the males were irradiated all produced egg cases with viable embryos. The 3000r pairs produced 52 cases containing 159 embryos of which 92 per cent (146) survived. The 9000r group yielded 93 cases with 270 embryos. Of these, 226 or 84 per cent survived to the veliger and protoconch stages. Thus, irradiation of males at levels of 3000 or 9000r appeared to have no significant effect upon fecundity and viability of embryos. Apparently, 18,000r affected fecundity little, e.g. 31 egg cases for irradiated drills versus 30 for the controls, but reduced the viability of embryos as only 34 per cent (30) survived to the veliger and protoconch stages. Table 4 and Fig. 7 indicate that egg case deposition from groups with irradiated males was similar in

pattern to the controls.

The two groups in which the females were irradiated produced no egg cases.

These results demonstrated that irradiation of females with the experimental dosages reduces their fecundity to zero. They also indicated that while irradiation of males alone at these levels does not reduce egg case production, a dose of 18,000r significantly reduces viability of embryos (Fig. 4).

Table 3

Viability and Egg Case - Embryo Totals

Experiment 2

<u>Mated Pairs</u>	<u>Total Egg Cases</u>	<u>Total Embryos</u>	<u>% Reaching Veliger & Protoconch</u>
15 ♂ 3000r. 15 ♀ 0	52	159	91.8
15 ♂ 9000r. 15 ♀ 0	93	269	83.7
15 ♂ 18000r. 15 ♀ 0	31	87	33.7
30 ♂ control 30 ♀ control	30	74	82.4

Irradiated ♀♀ produced no eggs

Table 4

Egg Case Production With Time

Experiment 2

Date	X ♂♂ & N ♀♀		X ♀♀	Controls	
	No. Cases	Accum. %		No. Cases	Accum. %
Sept. 23, 1962	6	3.4	No egg case deposition	-	-
26	15	11.9		-	-
28	3	13.6		-	-
29	8	18.2		2	6.7
Oct. 2	5	21.0		-	-
3	3	22.7		-	-
8	17	32.4		3	16.7
10	18	42.6		3	26.7
15	16	51.7		10	60.0
16	16	60.8		-	-
18	10	66.5		-	-
19	16	75.5		1	63.3
21	4	77.8		-	-
27	27	93.2		-	-
28	10	98.8		11	100.0
Nov. 2	2	100.0			

X - Irradiated

N - Non-irradiated

Experiment 3

In order to obtain more data, a third series of drills was irradiated on May 6, 1963. According to observations before and at the time of the experiment, this was slightly after the period of egg case deposition had begun. Copulation was probably continuing at a relatively high level. Observations of experimental animals were made at daily intervals and terminated on June 10, 1963, or 35 days after irradiation.

According to the data (Table 5) the control pairs (30 in this experiment as compared with 15 in the previous ones) produced 268 egg cases containing 1089 embryos. Of these, 96 per cent (1045) survived.

In the pairs in which only the males were irradiated, the egg case and embryo production (fecundity) were as high as, or higher than, in the control group. For example, at 3000r 183 egg cases containing 701 embryos were produced; at 9000r 152 egg cases bearing 498 embryos resulted; and, at 18,000r 261 egg cases bearing 979 embryos were spawned. Viability of 3000r and 9000r groups was not significantly lowered with 576 embryos, or 82.2 per cent, of the 3000r group, and 454 embryos, or 91.2 per cent, of the 9000r group surviving to the veliger and protoconch stages. However, embryo viability was lowered to a 57.1 per cent survival of those produced by the 18,000r group.

The pairs in which only the females were irradiated yielded the following:

- 1) 3000r - 20 egg cases containing 77 embryos with 13 embryos or 16.9 per cent surviving;
- 2) 9000r - no egg cases; and
- 3) 18,000r - six egg cases with 27 embryos and none surviving.

The group in which the males and females were both irradiated produced the following:

- 1) 3000r - 49 egg cases containing 187 embryos with 25 or 13.4 per cent surviving;
- 2) 9000r - 22 egg cases containing 99 embryos and no survival; and
- 3) 18,000r - 2 egg cases with 9 embryos and no survival.

This series indicates that irradiation of males is not effective in reducing fecundity at these levels and under these experimental conditions, whereas irradiating the females is effective. It also indicates that irradiation of males only, causes a significant reduction of viability at 18,000r but not at 3000 or 9000r (Fig. 5).

Because the experiment was begun after egg case deposition had started, irradiation of females did not stop egg case production completely, but it did reduce survival to zero in the 9000 and 18,000r groups and to very low levels (16.9 and 13.4 per cent) in the 3000r pairs.

The data represented in Fig. 8 again indicate that the rate of egg case production of pairs in which the females received no irradiation was nearly the same whether or not the males were irradiated (controls versus pairs in which only the males were irradiated). However, these pairs whose females were irradiated produced cases at

a much more rapid rate before they stopped completely, although the total number of cases was much lower (see also Table 6). Thus, irradiation of female U. cinerea after egg case deposition has begun appears to stimulate egg case production in such a way that the bulk of the total number of egg cases produced was reached in a shorter period of time.

Table 5

Viability and Egg Case - Embryo Totals

Experiment 3

<u>Mated Pairs</u>	<u>Total Egg Cases</u>	<u>Total Embryos</u>	<u>% Reaching Veliger & Protoconch</u>
15 ♂ 3000r. 15 ♀ 0	183	701	82.2
15 ♂ 9000r. 15 ♀ 0	152	498	91.2
15 ♂ 18000r. 15 ♀ 0	261	979	57.1
15 ♂ 0 15 ♀ 3000r.	20	77	16.9
15 ♂ 0 15 ♀ 9000r.	0	0	0.0
15 ♂ 0 15 ♀ 18000r.	6	27	0.0
15 ♂ 3000r. 15 ♀ 3000r.	49	187	13.4
15 ♂ 9000r. 15 ♀ 9000r.	22	99	0.0
15 ♂ 18000r. 15 ♀ 18000r.	2	9	0.0
30 ♂ control 30 ♀ control	268	1089	96.0

Table 6

Egg Case Production With Time

Experiment 3

Date	X ♂♂ & N ♀♀			N ♂♂ & X ♀♀			X ♂♂ & X ♀♀			Controls		
	No.	Cases	Accum. %	No.	Cases	Accum. %	No.	Cases	Accum. %	No.	Cases	Accum. %
May 9, 1963	12		2.0	-		-	-		-	-		-
11	-		2.0	-		-	-		-	9		3.4
12	11		3.9	-		-	-		-	5		5.3
13	-		3.9	-		-	-		-	3		6.4
14	16		6.6	-		-	-		-	6		8.6
15	27		10.8	-		-	-		-	6		10.8
16	17		13.7	6		23.1	19		26.0	9		14.2
17	63		24.3	16		84.6	14		45.2	7		16.8
20	21		27.8	-		-	40		100.0	31		28.4
22	57		37.4	4		100.0	-		-	41		43.7
23	27		41.9							12		48.2
24	14		44.2							-		48.2
27	71		56.1							15		53.8
30	64		66.8							16		59.8
June 3	93		82.4							26		69.5
5	5		83.2							11		73.6
6	7		84.4							-		73.6
10	93		100.0							71		100.0

X - Irradiated

N - Non-irradiated

Discussion

As previously mentioned, the time of the year that the drills were irradiated in relation to their egg case deposition cycle was probably the factor causing differences in the results of the three experiments. In experiment 1, the irradiation procedure was performed 17 days after the cycle had begun; in experiment 2, it was carried out four weeks before any deposition started; and in the last experiment, irradiation came only four days after the cycle had begun.

In experiment 1, irradiated females produced more egg cases than those in the other two. However, these females completed over 90 per cent of their total egg case production after only four days, whereas the non-irradiated females produced egg cases over a 21 day period at a fairly constant rate. Apparently, this 90 per cent production in the first four days came from egg case formation processes which had begun before irradiation. In experiment 2, no egg cases were deposited by irradiated females since the relatively early irradiation came before these processes were initiated. In the last experiment, much fewer egg cases were deposited by treated females than in experiment 1, since the irradiation was administered closer to the start of the deposition cycle. In the two experiments in which irradiated females produced egg cases, their total production was completed in a much shorter time than non-irradiated females. Again, this illustrates the effect of irradiation upon female fecundity.

These experiments also show the damaging effect of high-energy x-rays on extremely early zygote stages. The highest embryo survival rate at the 9000r dose was 31.9 per cent. When adult females received 18,000r, no embryos survived.

These experiments were originally designed to discover whether or not irradiation of male drills could effectively reduce the viability and/or fecundity of drill populations to the extent that the procedure could be used to control these predator animals. As previously mentioned, only by inducing dominant lethal genes in the male drill could such a control technique be used practically. In all but the first experiment, the effects of dominant lethal genes were indicated by the significantly low survival of embryos produced in containers in which the adult males received 18,000r.

As in egg case production, the relative time of irradiation in respect to the deposition cycle was apparently important in the reduction of embryo viability. Several inferences might be made to explain the absence of a dominant lethal effect in experiment 1. It is possible that a great many eggs within the females were already fertilized before irradiation. Another presumption might be that post-irradiation copulation was less frequent since the females were already highly inseminated. Lastly, it might have been physically impossible for the irradiated sperm to compete with the normal sperm because of the manner of storage by the females. But judging from the second and third experiments, this last possibility appears improbable.

The second experiment displayed the greatest dominant lethal effect of the three. Again, the relatively early date of irradiation might have given the treated sperm more time to compete in the fertilization of eggs. Also, more post-irradiation copulation could have occurred since much of the sperm previously stored by the females were depleted by the spring deposition cycle.

Further experimentation should be conducted to find out whether or not this irradiation procedure will be practical in the control of oyster drills. Work should be done using dosages closer to the 18,000r level to find the most efficient dose. Discovering an inexpensive method of collecting drills in the field, and designing a portable gamma ray source able to produce the necessary dosages will probably be the most difficult problems to be solved. Gamma rays would be used, since they are more practical and have essentially the same effect on the animals as x-rays (Bushland and Hopkins, 1953).

Summary

These experiments were conducted to determine whether x-rays could be used to control the oyster drill, Urosalpinx cinerea.

The following conclusions were arrived at:

- 1) High-energy x-rays applied to females before the deposition cycle begins eliminate egg case production.
- 2) A dosage of 18,000r when applied to adult male drills before the deposition cycle begins reduces embryo survival significantly.
- 3) High-energy x-rays have a marked effect on early zygote stages in that an extremely low percentage of these embryos survive.

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APPENDIX

Figure 3. Embryo survival, experiment 1.

X= irradiated; N= non-irradiated.

*** No survival of embryos from ♀♀
irradiated at 18,000r.**

Figure 3

Embryo Survival

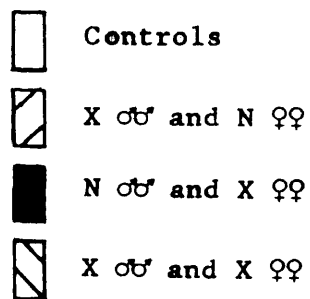
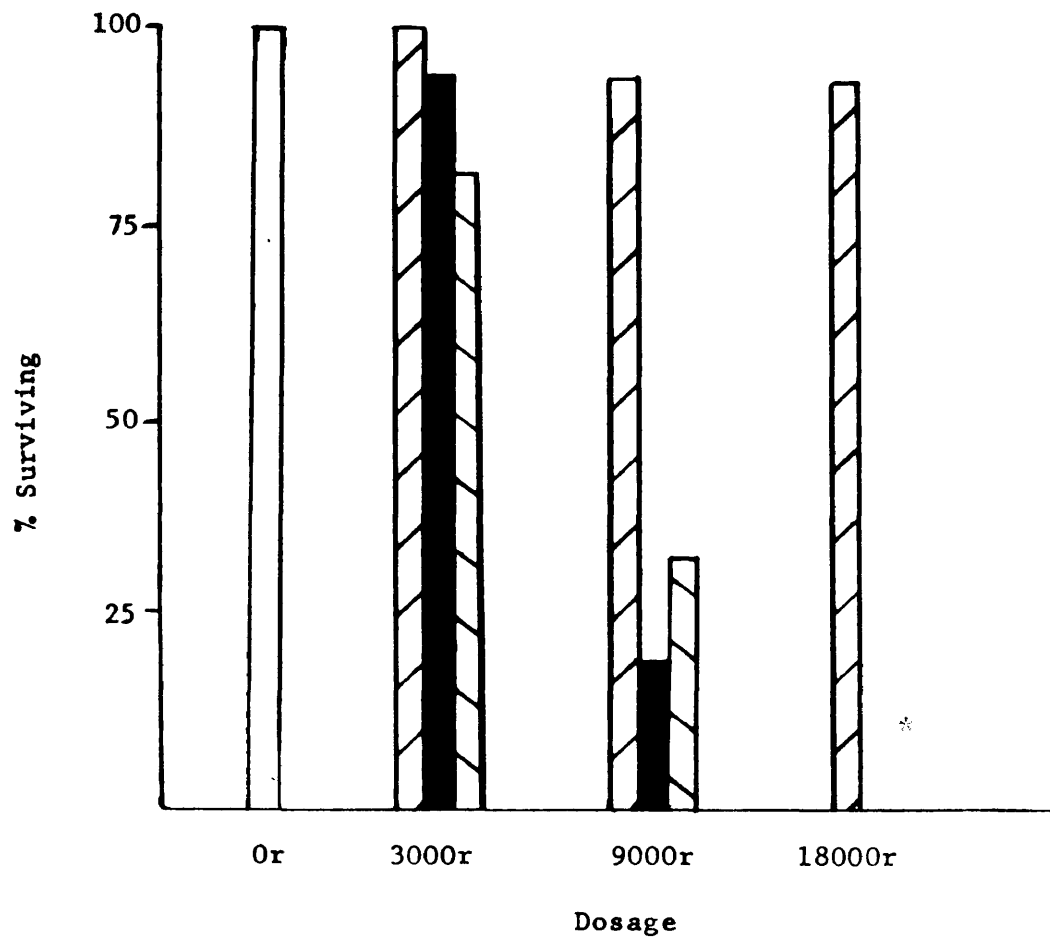


Figure 4. Embryo survival, experiment 2.

X= irradiated; N= non-irradiated.

Figure 4

Embryo Survival

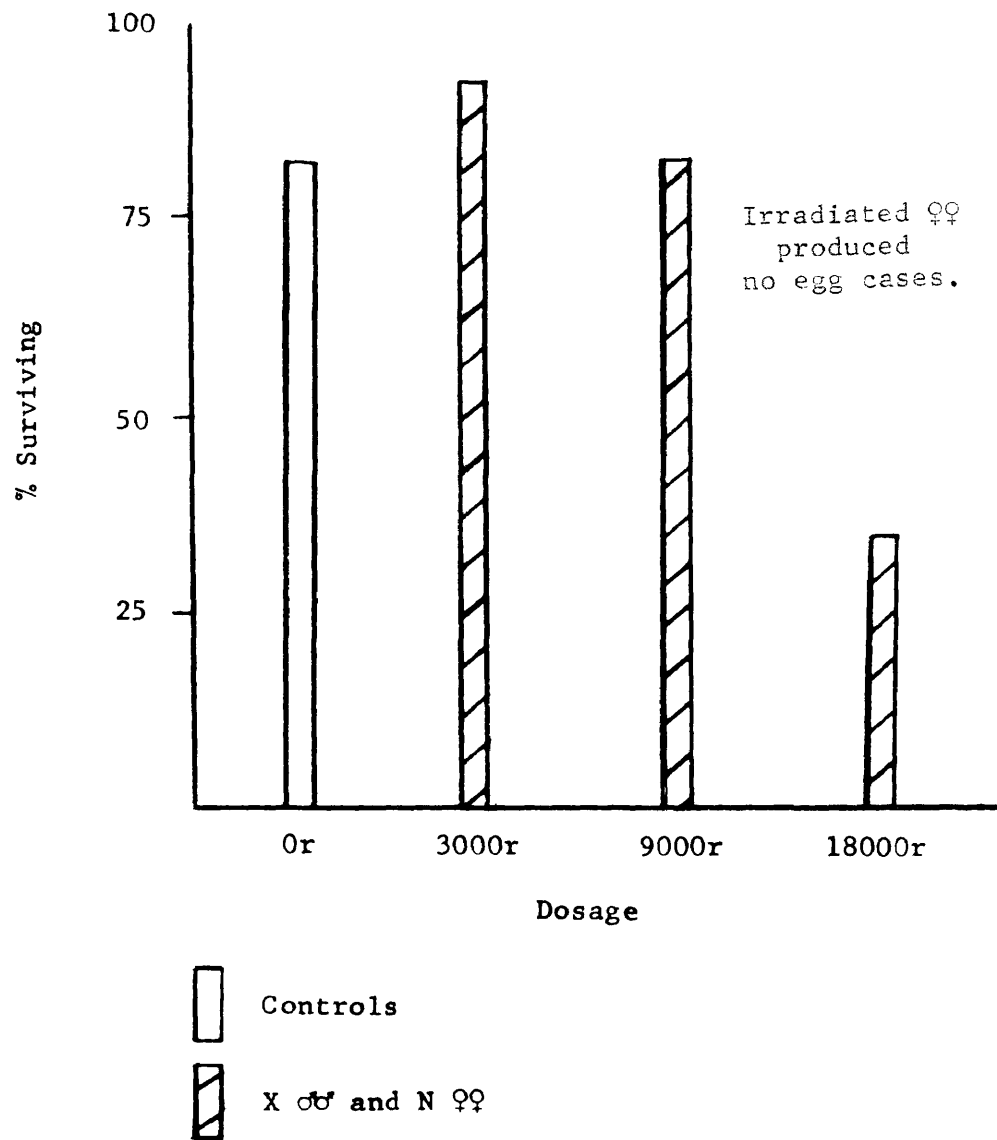


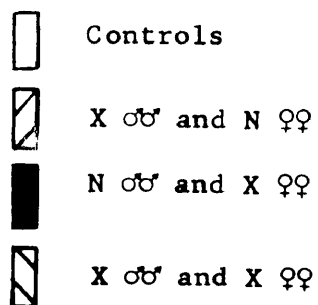
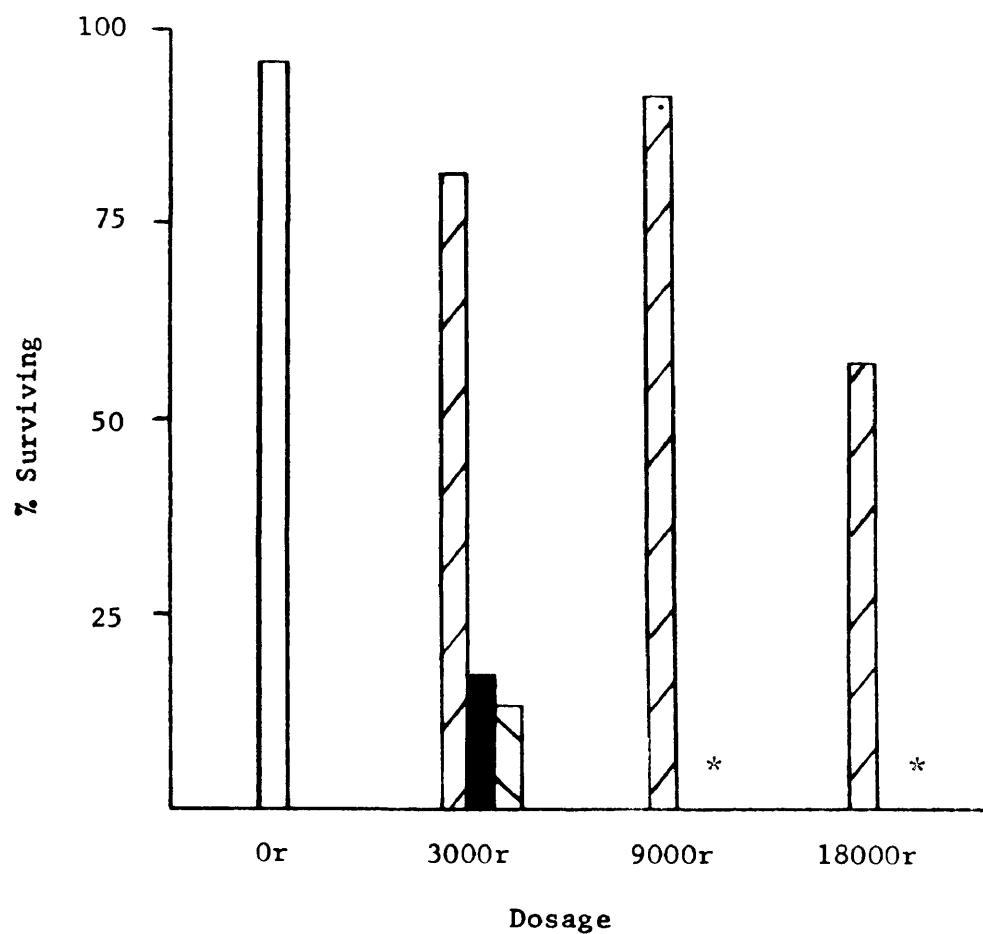
Figure 5. Embryo survival, experiment 3.

X= irradiated; N= non-irradiated.

*** No survival of embryos from ♀♀
irradiated at 18,000r and 9000r.**

Figure 5

Embryo Survival

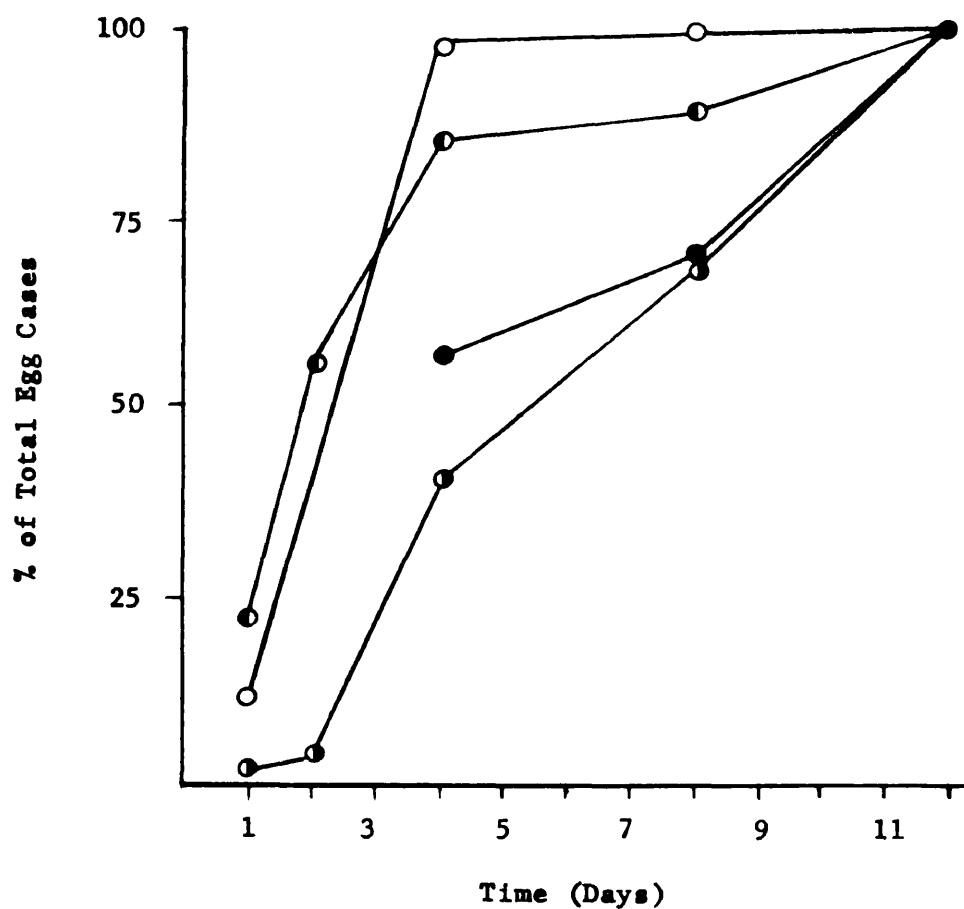


**Figure 6. Per cent of total egg case production with time,
experiment 1.**

X= irradiated; N= non-irradiated.

Figure 6

Egg Case Production



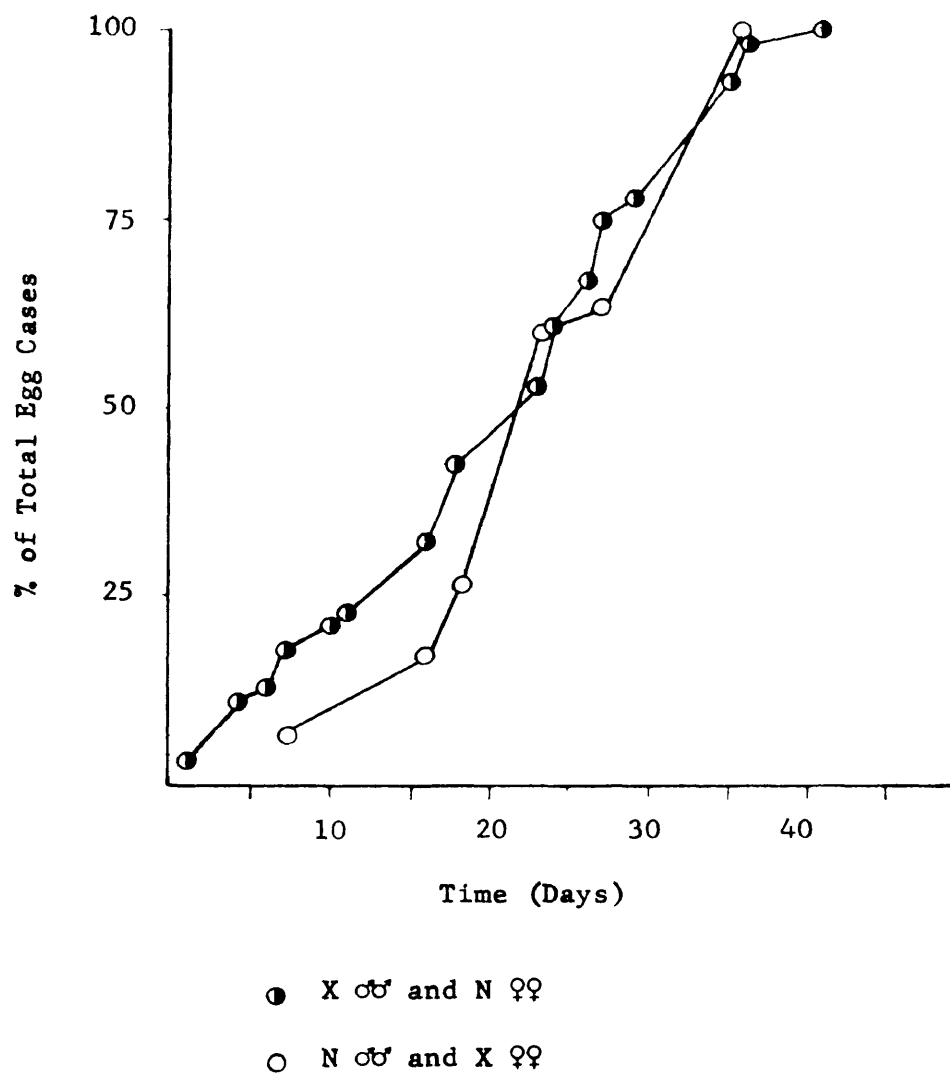
- X ♂ and N ♀
- ◐ N ♂ and X ♀
- X ♂ and X ♀
- Controls

**Figure 7. Per cent of total egg case production with time,
experiment 2.**

X= irradiated; N= non-irradiated.

Figure 7

Egg Case Production

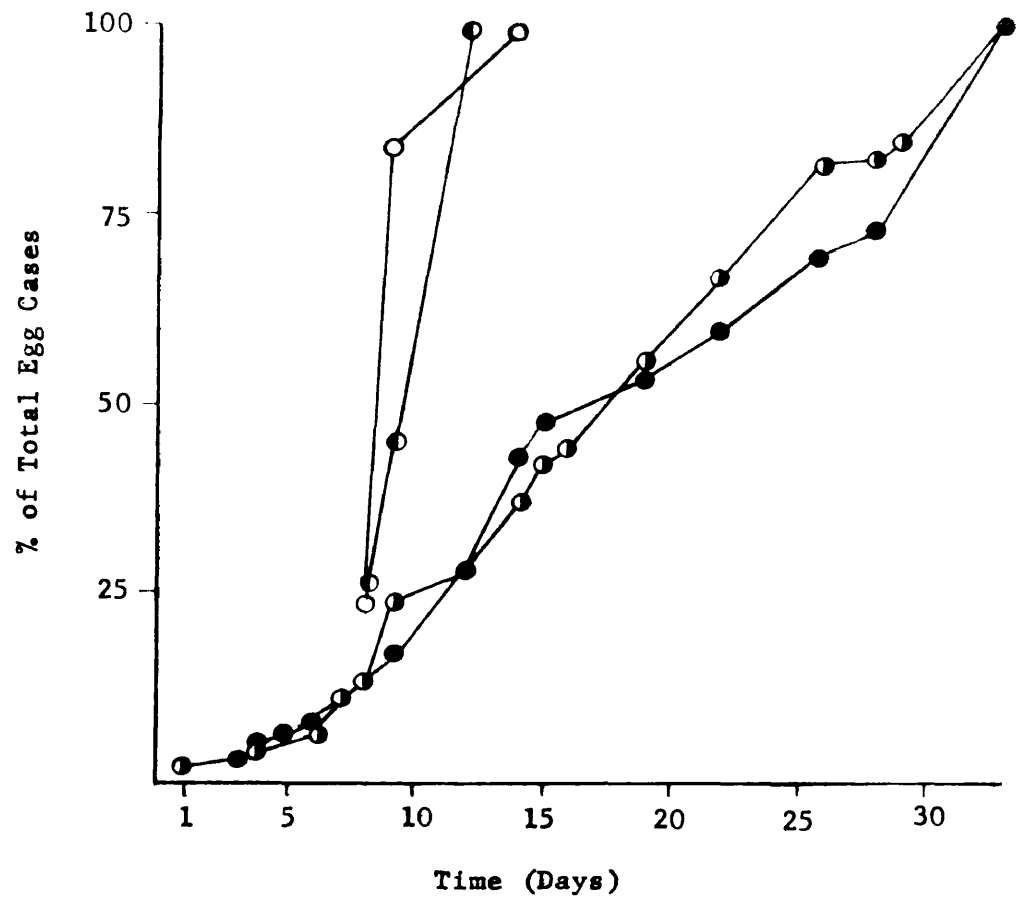


**Figure 8. Per cent of total egg case production with time,
experiment 3.**

X= irradiated; N= non-irradiated.

Figure 8

Egg Case Production



- X ♂♂ and N ♀♀
- N ♂♂ and X ♀♀
- ◐ X ♂♂ and X ♀♀
- Controls